Aus den Medizinischen Kliniken und Polikliniken
Klinik D der Universität Düsseldorf
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EFFECT OF THYROID HORMONES ON THE ACTIVITIES OF HEPATIC ALCOHOL METABOLIZING ENZYMES

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EFFECT OF THYROID HORMONES ON THE ACTIVITIES OF HEPATIC ALCOHOL METABOLIZING ENZYMES

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Summary

Treatment with thyroxine or triiodothyronine for 7 days in order to simulate a hyperthyroid state results in an enhanced activity of the microsomal ethanol oxidizing system. Conversely, a decrease of hepatic alcohol dehydrogenase activity was observed under these experimental conditions, whereas hepatic catalase activity remained unchanged. These findings suggest that if chronic ethanol consumption simulates a "hyperthyroid hepatic state", increased rates of ethanol metabolism observed following prolonged alcohol intake might therefore be attributed at least in part to an induction of microsomal ethanol oxidizing system activity in the liver.

The hepatocyte contains three different enzymes capable of oxidizing ethanol to acetaldehyde in vitro: alcohol dehydrogenase (ADH) of the cytosol or the soluble fraction of the cell (1, 2), the microsomal ethanol oxidizing system (MEOS) in the endoplasmic reticulum (2 - 5) and catalase in the peroxisomes (6). A variety of studies have suggested that ADH and MEOS fully account for rates of ethanol metabolism (2, 7), whereas the role of catalase is negligible (2, 8). Following chronic alcohol consumption an adaptive increase of MEOS activity occurs which was considered to contribute to increased rates of alcohol metabolism commonly observed after chronic alcohol consumption (7, 9, 10).

It has been reported, however, that some similarities may exist in the livers of alcohol treated animals compared to those given thyroid hormones especially since hepatic uptake of thyroxine was found to be increased following chronic alcohol consumption (11). Based upon these findings it has been

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suggested that chronic alcohol consumption results in a "hyperthyroid hepatic state", which was considered to be responsible for increased rates of ethanol metabolism (12).

In the present study the effect of thyroid hormones on the activities of the three hepatic enzymes capable of oxidizing ethanol in vitro was therefore investigated to elucidate the underlying mechanism for increased ethanol metabolism under these conditions.

Material and Methods

Female Sprague-Dawley rats with a body weight of 150 - 250 g were obtained from Zentralinstitut für Versuchstierzucht, Hannover, West Germany. They were fed Altromin-R chow ad libitum and had free access to tap water. L-Thyroxine (sodium salt from SERVA Feinbiochemica, Heidelberg, West Germany; 150 µg/100 g BW) and L-3,3',5-triiodothyronine (sodium salt from SERVA Feinbiochemica, Heidelberg, West Germany; 10 µg/100 g BW) dissolved in 0.5 ml alkaline solution (10 mM NaOH in 0.9 % NaCl) or the same amount of vehicle alone (controls) was injected s. c. daily for seven consecutive days. The animals were killed 24 h after the last injection.

The rats were decapitated, their livers in situ perfused with ice-cold 0.15 M KCl through the portal vein, excised, chilled, and a 25 % homogenate was prepared with the same solution. The homogenate was centrifuged at 10,000 g for 30 min, and the resulting supernatant was used for the subsequent preparation of washed microsomes and of liver cytosol (100,000 g supernatant) with an ultracentrifuge (13). Protein concentration was determined by the method of Lowry et al. (14).

Alcohol dehydrogenase activity was measured in the cytosol according to the method of Bonnichsen and Brink (15) with 0.1 M phosphate buffer (pH 7.4) and expressed in nmoles NADH formed per min per mg protein.

Microsomal ethanol oxidizing system (MEOS) activity was determined in the main chamber of 50 ml Erlenmeyer flasks with center wells containing 0.6 ml of semicarbazide hydrochloride (15 mM in 0.1 M phosphate buffer, pH 7.4). Microsomes (3 mg protein/flask) were incubated with ethanol (50 mM), sodium azide (0.1 mM) and a NADPH generating system (13, 16). The activity was expressed as nmoles acetaldehyde formed per min per mg microsomal protein.

Catalatic activity of catalase was determined in the 25 % liver homogenate by measuring the decrease in absorption at 240 nm following addition of H_2O_2 and expressed as units per g liver wet weight according to Tuck (17).

Results

To study the effect of a hyperthyroid state on the activities of various alcohol metabolizing enzymes in the liver, female rats were treated with thyroid hormones, and subcellular fractionation of the hepatocyte was subsequently performed.

Table 1

ADH, MEOS AND CATALASE ACTIVITIES AFTER TREATMENT WITH L-THYROXINE (T_4) OR TRIIODOTHYRONINE (T_3)

	ENZYME ACTIVITY		
Treatment	ADH	MEOS	Catalase
Controls	5.0 ± 0.3	4.0 ± 0.2	1.55 ± 0.1
T_4	2.4 ± 0.3 ($p < 0.001$)	5.8 ± 0.6 ($p < 0.005$)	1.63 ± 0.1 NS
T_3	2.5 ± 0.4 ($p < 0.001$)	5.0 ± 0.1 ($p < 0.025$)	1.77 ± 0.2 NS

Female rats were treated for 7 days by s. c. injection of T_4 (150 µg/100 g BW) or T_3 (10 µg/100 g BW) dissolved in 0.5 ml alkaline solution (10 mM NaOH in 0.9 % NaCl). The controls received the same volume of the vehicle. ADH activity was determined in the cytosol and expressed as nmoles NADH formed per min per mg protein. MEOS activity was determined in washed microsomes and expressed as nmoles acetaldehyde formed per min per mg protein. Catalatic activity of catalase was determined in the 25 % homogenate and expressed as Lück units x 10^3 per g liver. The values are derived from 18 experimental animals and represent means ± SEM.

Compared to controls, treatment with L-thyroxine (150 µg/100 g BW) for 7 days resulted in a significant decrease of alcohol dehydrogenase activity by 52 % ($p < 0.001$), as measured in the hepatic cytosol (Table 1). Similarly, a decrease by 50 % ($p < 0.001$) was observed in animals treated with triiodothyronine for 7 days (Table 1). Conversely, under these experimental conditions a pronounced increase of MEOS activity was observed after both T_4 and T_3 treatment by 45 % ($p < 0.025$) and 25 % ($p < 0.005$), respectively, when determined in washed microsomes (Table 1). Finally, when the catalatic activity of catalase was measured in the 25 % homogenate after the same treatment with L-thyroxine or triiodothyronine, catalatic activity remained virtually unchanged when compared to controls receiving the vehicle only (Table 1).

Table 2

EFFECT OF IN VITRO ADDITION OF T_4 OR T_3 ON RAT LIVER ADH, MEOS AND CATALASE ACTIVITIES

Assay Conditions	ENZYME ACTIVITY		
	ADH	MEOS	Catalase
No addition	4.77	4.47	1.52
T_4 (ng)			
100	4.75	4.74	1.14
1.000	4.38	4.63	1.29
10.000	4.28	4.75	1.05
T_3 (ng)			
100	4.19	4.81	1.21
1.000	4.38	4.91	1.19
10.000	4.44	4.90	1.28

Cytosol, washed microsomes and 25 % liver homogenate were obtained from untreated rats. When indicated, L-thyroxine (T_4; sodium salt) or triiodothyronine (T_3; sodium salt) was preincubated with the enzyme for 5 min before the reaction was started. The final incubation volume was 3.0 ml. ADH activity was expressed as nmoles NADH formed per min per mg protein, MEOS activity as nmoles acetaldehyde formed per min per mg microsomal protein and catalatic activity of catalase as Lück units x 10^{-3} per g liver. The results are means of two experiments.

Since prolonged in vivo treatment of rats with T_4 or T_3 resulted in a striking decrease of ADH activity, a simultaneous induction of MEOS activity and an unaltered catalatic activity of catalase (Table 1), the question arose whether the in vitro addition of these hormones may influence the activities of these three enzymes. T_4 or T_3 were therefore added at various amounts ranging from 100 ng to 10.000 ng and preincubated with the enzyme for 5 min before the reaction was started. The control incubations were carried out without addition of the hormones. Compared to control incubations, T_4 at low doses failed to inhibit ADH activity, whereas higher amounts resulted in a slight decrease of activity (Table 2). T_3 at concentrations

ranging from 100 ng to 10.000 ng exhibited a slight inhibitory effect on ADH activity (Table 2). Moreover, in vitro addition of T_4 or T_3 was without any effect on MEOS activity, irrespective whether low or high amounts of thyroid hormones were employed (Table 2). Finally, catalatic activity of catalase was slightly inhibited in the presence of T_4 or T_3 (Table 2). These experiments therefore show that the in vitro addition of thyroid hormones failed to affect ADH, MEOS and catalase activities to a major extent.

Discussion

There is considerable debate whether increased rates of ethanol metabolism following chronic alcohol consumption are due to alteration of hepatic alcohol metabolizing enzymes activities such as MEOS (7, 9, 10) or to a "hyperthyroid hepatic state" (11). The present study shows that a hyperthyroid state produced by administration of T_4 or T_3 significantly decreases ADH activity and induces MEOS activity, without altering catalase activity (Table 1). These findings therefore suggest that if chronic ethanol consumption simulates a "hyperthyroid hepatic state", increased rates of ethanol metabolism observed following prolonged alcohol intake might therefore be attributed at least in part to an induction of MEOS activity in the liver.

The observation that chronic alcohol consumption resulted in an increased hepatic thyroxine uptake (11) associated with an enhanced activity of hepatic ATP-ase (18) and increased hepatic oxygen consumption (19) led to the hypothesis that a "hyperthyroid hepatic state" may be created under these conditions. This was considered to be responsible for increased rates of ethanol metabolism through increased mitochondrial NADH reoxidation (12) the rate limiting step in the ADH mediated ethanol metabolism (20, 21). Other investigators, however, observed increased alcohol clearance despite normal ATP-ase activity (22). Similarly, O_2 uptake was found by others not to be increased following chronic alcohol consumption (22, 23). Moreover, it is generally accepted that chronic alcohol intake results in mitochondrial injury both by electron microscopical (24) as well as biochemical assessment (25), casting doubts on

a mitochondrial mechanism of increased ethanol clearance. Finally, it has been reported that the acceleration of ethanol oxidation at high ethanol concentrations and a major part of the adaptative increase in ethanol metabolism after chronic ethanol intake can be attributed to the increase of MEOS activity (10).

The present study therefore suggests that irrespective whether chronic alcohol consumption may create a "hyperthyroid hepatic state" or not, increased rates of ethanol metabolism can be ascribed at least in part to an induction of MEOS activity.

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Teil II:

HEPATIC MICROSOMAL ETHANOL OXIDIZING SYSTEM (MEOS): INCREASED ACTIVITY FOLLOWING PROPYLTHIOURACIL ADMINISTRATION

Fernando Moreno, Alexander S. Petrides, Edgar Heinen, Georg Strohmeyer, and Rolf Teschke

Abstract

Treatment for seven days with the thyreostatic drug propylthiouracil (5 mg/100g BW) resulted in a hypothyroid hepatic state as shown by the marked decreased hepatic content of thyroxine and triiodothyronine. This regimen led to an enhanced activity of the microsomal ethanol oxidizing system whereas the activities of alcohol dehydrogenase and catalase remained unchanged. Moreover, a hyperthyroid hepatic state achieved following the administration of L-thyroxine (150 µg/100g BW) or L-3, 3', 5-triiodothyronine (10 µg/100g BW) for seven days resulted in a similar increased activity of the microsomal ethanol oxidizing system. Under these conditions, a decrease of alcohol dehydrogenase activity and an unaffected catalase activity was observed. These findings therefore show that the administration of either propylthiouracil or thyroid hormones results in an increased activity of the microsomal ethanol oxidizing system, suggesting that the underlying mechanism for the induction of the microsomal ethanol oxidizing system by propylthiouracil is independent of the action of thyroid hormones.

Chronic alcohol consumption leads to a variety of hepatic alterations such as alcoholic fatty liver (1), alcoholic hepatitis (2) and alcoholic liver cirrhosis (2). It is now generally accepted that the development of alcoholic liver injury is due to the action of alcohol itself or to one of its metabolites rather than to dietary imbalance (2, 3).

The main goal of treatment of alcoholic liver disease is to achieve complete abstinence of alcohol. Under these conditions, the alcoholic fatty liver is fully reversible, and some impro[illegible]ce observed in patients with alcoholic hepatitis (4) and liver cirrhosis (5, 6). In addition, a variety of drugs have been tested such as adrenocorticosteroids (7) and d-penicillamine (8) but no significant benefit was achieved. Recently, treatment with the thyreostatic drug propylthiouracil (PTU) was evaluated in patients with alcoholic liver disease, and some improvement was achieved by clinical and laboratorial assessment (9). The mechanism by which PTU exerts its effects is still unclear, since this drug also exhibits a protective effect with respect to the hepatotoxicity elicited by carbon-tetrachloride (10) or acetaminophen (11, 12).

Since PTU has been used in the treatment of alcoholic liver disease (9), the question arose whether PTU might alter the activities of alcohol metabolizing enzymes in the liver. Therefore, following treatment with PTU the activities of alcohol dehydrogenase (ADH), the microsomal ethanol oxidizing system (MEOS) and catalase were determined, all of which are capable of oxidizing ethanol to acetaldehyde in vitro (13). A variety of studies provided strong evidence that both ADH and MEOS fully account for rates of ethanol metabolism in intact liver tissue (14) and in vivo (15, 16, 17) whereas the role of catalase is negligible (13, 18).

MATERIALS AND METHODS

Reagents

The hormones, chemicals and enzymes were obtained from the following sources: T_4 (L-thyroxine sodium salt), T_3 (L-3, 3', 5-triiodothyronine sodium salt), PTU (6-n-Propyl-2-thiouracil) and DL-sodium isocitrate from SERVA Feinbiochemica, Heidelberg, West Germany; NAD^+ (grade II), $NADP^+$ and isocitric dehydrogenase (grade II) from Boehringer Mannheim, West Germany; T_4

(L-thyroxine ^{125}I), T_3 (L-3, 3', 5-triiodothyronine ^{125}I) and bovine albumin from Behring Werke AG, Marburg-Lahn, West Germany.

Animal treatment

Female Sprague-Dawley rats with a body weight of 150-250g were obtained from Zentralinstitut für Versuchstierzucht, Hannover, West Germany. The animals were fed Altromin-R chow ad libitum and had free access to tap water. They were divided into groups of 6 rats each and received either 6-n-Propyl--2-thiouracil (PTU) alone or in combination with L-thyroxine (T_4) or L-3, 3', 5--triiodothyronine (T_3). Two additional groups were given T_4 or T_3 only. All animals received for seven days 1.0 ml olive oil by gastric tube and 0.5 ml of an alkaline solution (10 mM NaOH in 0.9% NaCl; pH 11.6) subcutaneously. Six of these animals served as controls. When indicated, PTU (5 mg/day/100g BW) was incorporated into the olive oil, and the alkaline solution was used as a vehicle for T_4 (150 µg/day/100g BW) or T_3 (10 µg/day/100g BW).

Preparation of subcellular fractions

Twenty four hours after the last application the rats were killed by decapitation. The liver was perfused in-situ with ice-cold 0.15 M KCl and surgically removed. Subsequently , the livers were minzed using scissors, and after chilling a 25% homogenate was prepared with 0.15 M KCl using a Teflon-glass homogenizer. All the following steps were carried out at 0-4°C. The 25% liver homogenate was spun at 10,000 g for 30 min, and the supernatant was then centrifuged at 105,000 g for another 30 min. The resulting supernatant corresponds to the cytosolic fraction and was used for the determination of the alcohol dehydrogenase (ADH) activity. The pellet was resuspended and again spun at 105,000 g for another 30 min. The pellet consisted of washed microsomes and was used as enzyme source for the determination of the microsomal ethanol oxidizing system (MEOS) activity after resuspension (19).

Hormone determinations

The hepatic content of T_4 and T_3 was estimated in the 25% liver homogenate after extraction of T_4 and T_3 (20). The recovery of the acid butanol ammonia extraction was assayed by addition of T_4 or T_3 labeled with ^{125}I in each extract. It was found to be about 90% for T_3 and 85% for T_4, and was taken into account when calculating the hepatic thyronine concentration. In order to prevent

deiodination, propylthiouracil (10^{-4} M) was added to the homogenates. After evaporation to dryness the liver extracts were dissolved and the content of T_4 or T_3 was measured radioimmunologically (21).

Biochemical determinations

Alcohol dehydrogenase (ADH) activity was assayed in the hepatic cytosol by monitoring the reduction of NAD^+ at 340 nm in the presence of 50 mM ethanol with 0.1 M phosphate buffer (pH 7.4) as described before (13). The activity was expressed as nmoles NADH formed per min per mg cytosolic protein.

Microsomal ethanol oxidizing system (MEOS) activity was measured with washed microsomes (3 mg of microsomal protein per flask) which had been preincubated with 50 mM ethanol for 5 min at 37°C (22). The reaction was initiated by adding the NADPH generating system (0.4 mM $NADP^+$, 8 mM sodium isocitrate and 0.34 unit/ml of isocitric dehydrogenase). The incubations were carried out in a final volume of 3.0 ml containing 1.0 mM disodium-EDTA, 5.0 mM $MgCl_2$ and 0.1 mM sodium azide in 0.1 M phosphate buffer (pH 7.4) and terminated after 0, 5 and 10 min of incubations time by adding 0.5 ml of 70% (w/v) trichloroacetic acid. After an overnight diffusion period, the acetaldehyde formed by the oxidation of ethanol was measured as its semicarbazone derivative in a Zeiss DM 4 spectrophotometer. The activity was expressed as nmoles acetaldehyde formed per min per mg microsomal protein.

Catalase activity was measured in the 25% liver homogenate by following the disappearance of added H_2O_2 at 240 nm and expressed as units per g liver wet weight according to Lück (23).

Protein concentration was measured in the cytosolic and microsomal fraction of the hepatocyte, using bovine albumin as standard (24).

Statistical analyses

The results obtained are expressed as means $\pm$ SEM, and the significance of the differences was assessed by the Students t-test for unpaired data.

RESULTS

Hepatic content of L-thyroxine (T_4)

The hepatic content of T_4 was determined following the administration of propylthiouracil (PTU) and/or thyroid hormones for seven days (Table 1). Compared

to controls, the administration of PTU resulted in a significant decrease of the hepatic content of T_4 by 63% ($p<0.05$), suggesting a blockade of thyroidal synthesis of T_4. When compared to either the controls or to the PTU-treated rats, the combined application of PTU and T_4 resulted in a marked increase of the hepatic T_4 content. This increase, however, was much more striking compared to the effect observed after the administration of T_4 alone (Table 1), indicating an effective inhibition of the conversion of T_4 to T_3 by PTU. Moreover, compared to controls the administration of T_3 failed to significantly alter the hepatic content of T_4, irrespective whether T_3 was applicated alone or with PTU.

Hepatic content of L-3, 3', 5-triiodothyronine (T_3)

Compared to controls, treatment with PTU (Table 1) for seven days resulted in a marked decrease of the hepatic content of T_3 by 86% ($p<0.001$), indicating a blockade of thyroid hormone synthesis and of peripheral conversion of T_4 to T_3. Taking into account the low hepatic contents of T_4 and T_3 following the administration of PTU, the animals can be considered to exhibit a hypothyroidal hepatic state. Compared to controls, PTU administration combined with T_4 failed to alter the hepatic content of T_3, whereas the application of T_4 alone resulted in a striking enhancement of the T_3 liver content by 340% ($p<0.001$). Moreover, compared to controls the administration of T_3 led to a marked increase of the hepatic content of T_3 by 280% ($p<0.001$) which was much more pronounced when PTU was administered simultaneously. These findings suggest an effective inhibition of T_3 degradation by PTU.

Alcohol dehydrogenase (ADH)

When compared to control animals, ADH activity in the cytosolic fraction of the hepatocyte was found not to be altered following the administration of PTU for seven days (Table 2). In contrast, treatment with PTU together with T_4 or T_3 resulted in a significant decrease of ADH activity by 28% ($p<0.05$) and 51% ($p<0.001$), respectively (Table 2). Similarly, a significant decrease of ADH activity by 53% ($p<0.001$) and 50% ($p<0.001$) could be observed following the administration of T_4 or T_3, respectively (Table 2). It is evident from these data that a decrease of ADH activity observed following the administration of either T_4 or T_3 (Table 2) is associated with the creation of a hyperthyroid hepatic state (Table 1). On the other hand, a hypothyroid hepatic state achieved following

the application of PTU (Table 1) was incapable of altering ADH activity (Table 2).

Microsomal ethanol oxidizing system (MEOS)

Compared to controls, the administration of PTU for seven days resulted in a significant increase of MEOS activity by 54% ($p<0.001$) (Table 3). When the administration of PTU was combined with T_4 or T_3, an increase of MEOS activity was observed by 80% ($p<0.001$) and 90% ($p<0.001$), respectively (Table 3). This increase, however, was much more pronounced than the one observed following the administration of T_4 or T_3 alone (Table 3), suggesting an additive effect of both PTU and thyroid hormones on MEOS activity. Of particular interest was the observation that an inductive effect of MEOS activity was observed after the creation of a hyperthyroid hepatic state following administration of thyroid hormones. This effect, however, was augmented rather than abolished by the administration of the thyreostatic drug PTU (Table 3).

Catalase

Compared to control animals no significant alteration of the catalatic activity of catalase was observed following the administration of PTU alone or in combination with thyroid hormones (Table 4).

DISCUSSION

The present study shows that administration of propylthiouracil (PTU) for seven days leads to a hypothyroid hepatic state as demonstrated by the marked decrease of the hepatic content of T_4 and T_3 (Table 1). This is in accord with several studies in vivo and in vitro showing that PTU inhibits the synthesis of thyroid hormones in the thyroid gland (25) and the extrathyroidal conversion of L-thyroxine (T_4) to the more active L-3,3',5-triiodothyronine (T_3) (26, 27). It has also been demonstrated that the conversion of T_4 to T_3 takes place in the microsomal fraction of the hepatocyte (28).

The administration of PTU leads not only to a hypothyroid hepatic state (Table 1) but also to an increase of MEOS activity (Table 3). Under these experimental conditions, the activities of ADH (Table 2) and catalase (Table 4) remained unchanged. Furthermore, when PTU treatment was combined with the administration of thyroid hormones a much higher increase of MEOS activity was

observed (Table 3), suggesting that both treatments may have their own inductive effect. Moreover, a hyperthyroid hepatic state achieved following the administration of T_4 or T_3 (Table 1) resulted in a significant increase of MEOS activity (Table 3) and in a decrease of ADH activity leaving the catalase activity unaffected (Table 4).

It has been reported that the application of triiodothyronine (T_3) to patients with an acute alcohol intoxication resulted in a striking increase of the metabolic rate of alcohol disappearance from the blood (29). This alteration occurred at high blood alcohol concentration (29), a finding which led to the conclusion that a non-ADH pathway might be involved (29). The present study showing a striking increase of MEOS activity following T_3 administration (Table 3) is in line with this concept that a non-ADH pathway such as the MEOS may have caused the acceleration of alcohol metabolism. On the oder hand, at low alcohol concentrations when the ADH pathway is operative, T_3 appears to have no effect on the rate of alcohol metabolism (30). In any event, the decrease of ADH activity following T_3 administration observed in the present study (Table 2) might mask the effect of the increased MEOS activity (Table 3) and explain why the application of T_3 may have no effect on the overall rate of alcohol metabolism (30).

The observation that the activity of the hepatic microsomal ethanol oxidizing system (MEOS) is increased following treatment with PTU (Table 3) is of particular interest since this drug has been used for the treatment of alcoholic liver disease (9). The decision for a therapeutical trial using PTU was based upon the hypothesis that chronic alcohol consumption may create a "hyperthyroid hepatic state" which was considered to be responsible for the development of alcoholic liver disease (31, 32). The hypothesis of a hyperthyroid hepatic state due to chronic ethanol consumption was based upon the finding that an acute dose of ethanol increases the uptake of thyroxine into the liver (31), but data on hepatic contents of thyroid hormones following chronic alcohol consumption have not yet been published. The hypothesis of a "hyperthyroid hepatic state" was also based on an increased hepatic oxygen consumption and enhanced ($Na^+ + K^+$) ATPase activity (31) following chronic alcohol consumption. However, other studies failed to find an increase of hepatic oxygen consumption (33, 34) or an increased ATPase activity (34) due to prolonged alcohol intake under conditions which nevertheless resulted in the development of alcoholic liver disease such

as alcoholic fatty liver (33, 34). It has also been suggested that an enhancement of the ATPase activity could be responsible for the increased rates of ethanol metabolism observed following chronic alcohol consumption through an acceleration of the alcohol dehydrogenase-dependent ethanol metabolism, due to an increased mitochondrial NADH reoxidation (35). However, the function of a mitochondrial mechanism for increased alcohol metabolism is unlikely since chronic alcohol consumption results in mitochondrial injury both by electronmicroscopical (36) as well as biochemical assessment (37). In fact, a variety of studies have suggested that the increased rates of ethanol metabolism following chronic alcohol consumption can be ascribed to the adaptive increase of MEOS activity observed after alcohol intake (38).

The observation that prolonged administration of thyroxine (T_4) or triiodothyronine (T_3) significantly increases MEOS activity (Table 3) suggest that if a "hyperthyroid hepatic state" may be created under certain experimental conditions following chronic alcohol consumption, the increased rates of ethanol metabolism can be ascribed at least in part to an induction of MEOS activity in the liver (39).

There is considerable dispute concerning the mechanism of the protective effect of PTU on the hepatotoxicity. T_3 has been incriminated in the pathogenesis of alcohol induced liver injury (32). However, treatment with the thyreostatic drug PTU led to an increase of the low serum T_3 levels which was associated with an improvement rather than deterioration in patients with severe alcoholic liver disease such as alcoholic hepatitis (40). Moreover, PTU exhibits a protective effect against the hepatotoxicity elicited by carbon-tetrachloride (10) or acetaminophen (11, 12) both of which are activated to toxic metabolites by microsomal enzymes. The mechanism by which PTU afforded protection on carbon-tetrachloride induced liver damage has not yet been fully defined. With respect to acetaminophen, an increased hepatic content of reduced glutathione and an enhanced glucuronidation were considered to play a significant role in the prevention of the hepatotoxic effect (12).

The present study therefore shows that treatment with thyroid hormones results in a hyperthyroid hepatic state (Table 1) which is associated with an increase of MEOS activity whereas the administration of the thyreostatic drug PTU had a similar effect on MEOS activity (Table 3). Since the latter treatment resulted in a

hypothyroid hepatic state (Table 1) it is suggested that the underlying mechanism of the induction of MEOS activity by treatment with PTU might be independent of the action of thyroid hormones.

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Table 1. EFFECT OF PTU AND THYROID HORMONES ADMINISTRATION ON THE HEPATIC CONTENT OF T_4 AND T_3

Treatment	Hepatic T_4		Hepatic T_3	
	ng/g liver tissue	p	ng/g liver tissue	p
Controls	16.5 ± 4.3		1.48 ± 0.25	
PTU	6.1 ± 2.8	<0.05	0.20 ± 0.07	<0.001
PTU + T_4	121.8 ± 17.5	<0.001	1.67 ± 0.40	N.S.
T_4	57.1 ± 12.1	<0.005	6.60 ± 1.05	<0.001
PTU + T_3	18.1 ± 2.5	N.S.	11.52 ± 1.44	<0.001
T_3	15.6 ± 2.9	N.S.	5.58 ± 0.91	<0.001

Female Sprague-Dawley rats were treated for seven days with propylthiouracil and/or thyroid hormones. The hepatic content of T_4 and T_3 was determined by radioimmunologic assay and expressed per g liver wet weight. The results are given as means $\pm$ SEM of 6 animals for each group.

Table 2. EFFECT OF PTU AND THYROID HORMONES ADMINISTRATION ON THE ACTIVITY OF ALCOHOL DEHYDROGENASE (ADH)

Treatment	ADH activity	
	nmoles NADH/min/mg protein	p
Controls	6.11 $\pm$ 0.61	
PTU	6.22 $\pm$ 0.83	N.S.
PTU + T_4	4.39 $\pm$ 0.57	<0.05
T_4	2.87 $\pm$ 0.38	<0.001
PTU + T_3	2.99 $\pm$ 0.43	<0.001
T_3	3.08 $\pm$ 0.40	<0.001

PTU or thyroid hormones were administered for seven days to female Sprague--Dawley rats. ADH activity was determined in the cytosol of the liver after ultracentrifugation. The values are calculated per mg cytosolic protein of the hepatocyte and represent means $\pm$ SEM of 6 animals.

Table 3. EFFECT OF PTU AND THYROID HORMONES ADMINISTRATION ON THE ACTIVITY OF THE MICROSOMAL ETHANOL OXIDIZING SYSTEM (MEOS)

Treatment	MEOS activity	
	nmoles acetaldehyde/min/mg protein	p
Controls	4.06 ± 0.15	
PTU	6.25 ± 0.32	<0.001
PTU + T_4	7.27 ± 0.25	<0.001
T_4	5.74 ± 0.42	<0.01
PTU + T_3	7.70 ± 0.24	<0.001
T_3	5.23 ± 0.20	<0.001

PTU and thyroid hormones were administered as indicated to female Sprague--Dawley rats for seven days. After centrifugation of the liver homogenate MEOS activity was determined in washed microsomes and expressed as nmoles acetaldehyde formed per min per mg microsomal protein. The data are given as means ± SEM of 6 animals each group.

Table 4. EFFECT OF PTU AND THYROID HORMONES ADMINISTRATION ON THE CATALATIC ACTIVITY OF CATALASE

Treatment	Catalase activity	
	Units x 10^3/g liver	p
Controls	1.96 $\pm$ 0.19	
PTU	2.10 $\pm$ 0.23	N.S.
PTU + T_4	2.06 $\pm$ 0.10	N.S.
T_4	2.06 $\pm$ 0.17	N.S.
PTU + T_3	1.65 $\pm$ 0.17	N.S.
T_3	2.04 $\pm$ 0.18	N.S.

Female Sprague-Dawley rats were treated for seven days with PTU and thyroid hormones. Catalatic activity of catalase was assayed in the 25% liver homogenate and expressed in Lück units x 10^3 per g liver wet weight. The values represent means $\pm$ SEM of 6 animals.

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Am 21. September 1951 wurde ich, Fernando Salvador Moreno, als Sohn des Rechtsanwaltes José Salvador Moreno und seiner Ehefrau Liselotte geb. Pfaffmann in Sao Paulo, Brasilien, geboren (Vater Brasilianer; Mutter deutscher Abstammung). Absolvierte das Humanistische Gymnasium des Colégio Visconde de Porto Seguro, Sao Paulo. Abgang mit Abitur 1969. Einberufung zum Militärdienst, zum Reserveoffiziers-Kursus auserwählt und am 28.11.1970 als Fähnrich des Brasilianischen Heeres abgegangen. 1971 Aufnahme des Medizinstudiums an der Medizinischen Fakultät Ribeirao Preto der Universität Sao Paulo. Abschluß im Jahre 1976. 1977 bis August 1978 war ich an den Universitätskliniken Sao Paulo, Abteilungen Gastroenterologie und Endoskopie, tätig. Seit 1.9.1978 beziehe ich ein Stipendium der Heinrich-Hertz-Stiftung des Landes Nordrhein-Westfalen und bin in der Medizinischen Klinik D der Universität Düsseldorf als wissenschaftlicher Assistent unter der Leitung von Herrn Prof. Dr. G. Strohmeyer beschäftigt.

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